Core Structure of the Outer Membrane Lipoprotein from *Escherichia coli* at 1.9 Å Resolution

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Introduction: The outer membrane of gram-negative bacteria is a complex structure composed of phospholipids, proteins, and polysaccharides that controls the permeability and helps maintain the shape and rigidity of the cell. The protein complement of the outer membrane includes a lipoprotein that is one of the most abundant proteins in bacteria. The lipoprotein has characteristic lipid modifications at an amino-terminal cysteine and can exist in a form bound covalently to the peptidoglycan through a carboxyl-terminal lysine.

Methods and Materials: Plasmid pLpp56 was constructed by PCR amplification from genomic *E. coli* DNA, followed by subcloning into the *Nde*I and *Bam*HI restriction sites of the vector pAED4. Lpp-56 was expressed in *E. coli* BL21(DE3)/pLysS using the T7 expression system. Proteins were purified from the soluble fraction to homogeneity by reverse-phase HPLC, using a Vydac C-18 preparative column. The identities of the peptides were confirmed by mass spectrometry, and all molecular weights were found to be within 2 daltons of the expected weight.

Results: The 56-residue polypeptide moiety of the lipoprotein, designated Lpp-56, folds into a stable, trimeric helical structure in aqueous solution. To gain a structural insight into the assembly of the lipoprotein, we determined the X-ray crystal structure of Lpp-56 at 1.9 Å resolution. The structure was solved by a molecular replacement approach and refined to a conventional *R*-factor of 21.4% and a free *R*-factor of 26.9% for data in the 15.0 to 1.9 Å resolution range. Three Lpp-56 molecules associate to form a homotrimer (**Figure 1**). The Lpp-56 trimer creates a cylinder, ~83 Å in length with circular cross-section of 21 to 24 Å. It is composed of three domains: (i) a long central three-stranded coiled-coil domain consisting of three parallel α helices wrapped in a gradual left-handed superhelix with a three-fold axis of symmetry, (ii) an amino-terminal capping motif that forms a well-defined network of multi-center hydrogen bonds, with an umbrella-shaped fold, and (iii) a novel hydrophobic helix-termination motif in which the aromatic rings of the carboxyl-terminal tyrosine residues interact with a three-fold axis of symmetry.

Conclusions: The 1.9 Å resolution crystal structure of Lpp-56 comprises a parallel three-stranded coiled coil including a novel alanine-zipper unit and two helix-capping motifs. The amino-terminal motif forms a hydrogen-bonding network anchoring an umbrella-shaped fold. The carboxyl-terminal motif uses puckering of the tyrosine side chains as a unique docking arrangement in helix termination. The structure provides an explanation for assembly and insertion of the lipoprotein molecules into the outer membrane of gram-negative bacteria and suggests a molecular target for antibacterial drug discovery.

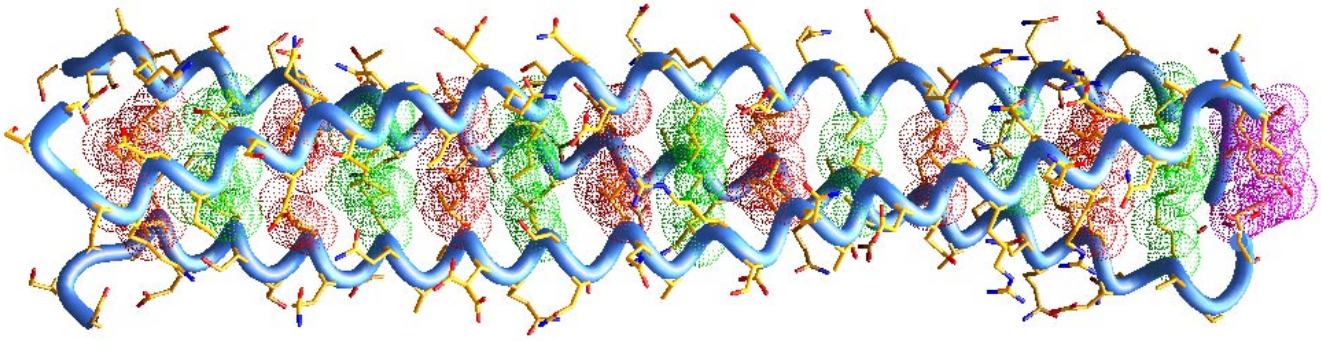

Figure 1. Side view of the Lpp-56 trimer. The van der Waals surfaces of residues at the ***a*** (red) and ***d*** (green) positions are superimposed on the helix backbone. The carboxyl-terminal tyrosine cap is shown in magenta.